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Dermal Inflammation Elicited by Synthetic Analogs of *Treponema pallidum* and *Borrelia burgdorferi* Lipoproteins

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The membrane lipoproteins of *Treponema pallidum* and *Borrelia burgdorferi* have potent immunostimulatory properties in vitro, implicating them as major inflammatory mediators in syphilis and Lyme disease. Recently, we reported that synthetic lipohexapeptide analogs (lipopeptides) of the lipoproteins could be used as surrogates for native spirochetal lipoproteins in immune cell activation studies in vitro. The present study was designed to evaluate the inflammatory properties of the lipopeptides in vivo and to correlate the cellular responses to these synthetic analogs with the histopathology of syphilis and Lyme disease. Lipopeptides corresponding to the 47-kDa major membrane lipoprotein of *T. pallidum* and the outer surface protein A of *B. burgdorferi* injected intradermally induced dose-dependent dermal inflammation in mice; the initial predominantly neutrophilic (mice) or heterophilic (rabbits) cellular infiltrates were followed by infiltrates consisting predominantly of mononuclear cells. The intradermal response of rabbits to the 47-kDa lipopeptide was strikingly similar to that observed for animals infected intradermally with *T. pallidum*. In all cases, lipopolysaccharide was substantially more potent as an inflammatory mediator than the spirochetal lipopeptides. In contrast to the lipopeptides, nonacylated hexapeptides elicited minimal or no dermal lesions in mice or rabbits, underscoring the importance of acyl modification to the inflammatory properties of the lipopeptides. This study provides the first in vivo evidence that the spirochetal lipoproteins/lipopeptides contribute to the immunopathogenesis of syphilis and Lyme disease.

Venereal syphilis and Lyme disease are chronic inflammatory disorders driven by the persistence of their respective etiologic agents, *Treponema pallidum* subsp. *pallidum* (*T. pallidum*) and *Borrelia burgdorferi* (26, 45, 47, 48). A comprehensive understanding of the pathogenesis of these two diseases depends on delineating features of these pathogens which contribute to their immunoevasiveness (34) and their ability to induce the protracted immunopathologic processes which culminate in clinical symptomatology (1, 16, 36). Characterization of spirochetal immunomodulators, the focus of the present study, is particularly important, given that neither *T. pallidum* nor *B. burgdorferi* contains lipopolysaccharide (LPS) (endotoxin) (20, 50), the potent inflammatory mediator of gram-negative bacteria (31).

A structural feature common to *T. pallidum* and *B. burgdorferi* is that the majority of their integral membrane proteins are lipid modified (9, 12, 42). Compelling data have now emerged supporting that these spirochetal lipoproteins are potent immunopotentiators. Treponemal and borrelial lipoproteins have been shown to activate monocytes/macrophages, B cells, and endothelial cells in vitro (1, 28, 29, 36, 39, 49, 56), suggesting that these molecules are inflammatory mediators in both syphilis and Lyme disease. More recently, we reported that synthetic lipohexapeptide analogs (lipopeptides) corresponding to the N termini of the native spirochetal lipoproteins could be used as lipoprotein surrogates in immune cell activation studies (16, 37). These lipopeptides, modeled after earlier studies of Bessler, Jung, and coworkers on the murein (Braun's) lipoprotein of *Escherichia coli* (21, 22), have been configured as *N*-palmitoyl-*S*-dipalmitoylglycerylcysteine-pentapeptides (16, 37).

Thus far, all of the data implicating spirochetal lipoproteins/lipopeptides as inflammatory mediators have been obtained from in vitro studies. The present investigations were undertaken to evaluate whether spirochetal lipopeptides exhibit analogous inflammatory properties in vivo. The basic design of the study was to perform histopathological analyses following intradermal challenge of laboratory animals with synthetic lipopeptides; intradermal challenge is an established method for assessing the inflammatory properties of bacterial immunomodulators (4, 11, 32). LPS was included in these studies, given the facts that (i) the dermal response to endotoxin has been extensively characterized in animal models (11) and (ii) LPS and bacterial lipoproteins/lipopeptides activate a similar spectrum of immune effector cells in vitro and evoke similar cytokine responses in monocytes/macrophages (16, 36, 37). A further rationale for studying the dermal responses to spirochetal lipopeptides derives from the fact that the skin is the natural portal of entry for both *T. pallidum* and *B. burgdorferi* and represents the site of initiation of the host's immune response.

MATERIALS AND METHODS

Peptides and lipopeptides. The lipopeptides and peptides used in this study corresponded to the N termini of the 47-kDa major membrane lipoprotein of *T. pallidum* (Cys-Gly-Ser-Ser-His-His) (54, 55) and the abundant outer surface protein A (OspA) of *B. burgdorferi* (Cys-Lys-Gln-Asn-Val-Ser) (6). Lipohexapeptides (average molecular weight, ca. 1,400) and nonacylated hexapeptides (average molecular weight, ca. 700) were synthesized and analyzed for purity by negative-ion fast atom bombardment mass spectrometry, matrix-assisted laser desorption mass spectrometry, and thin-layer chromatography as

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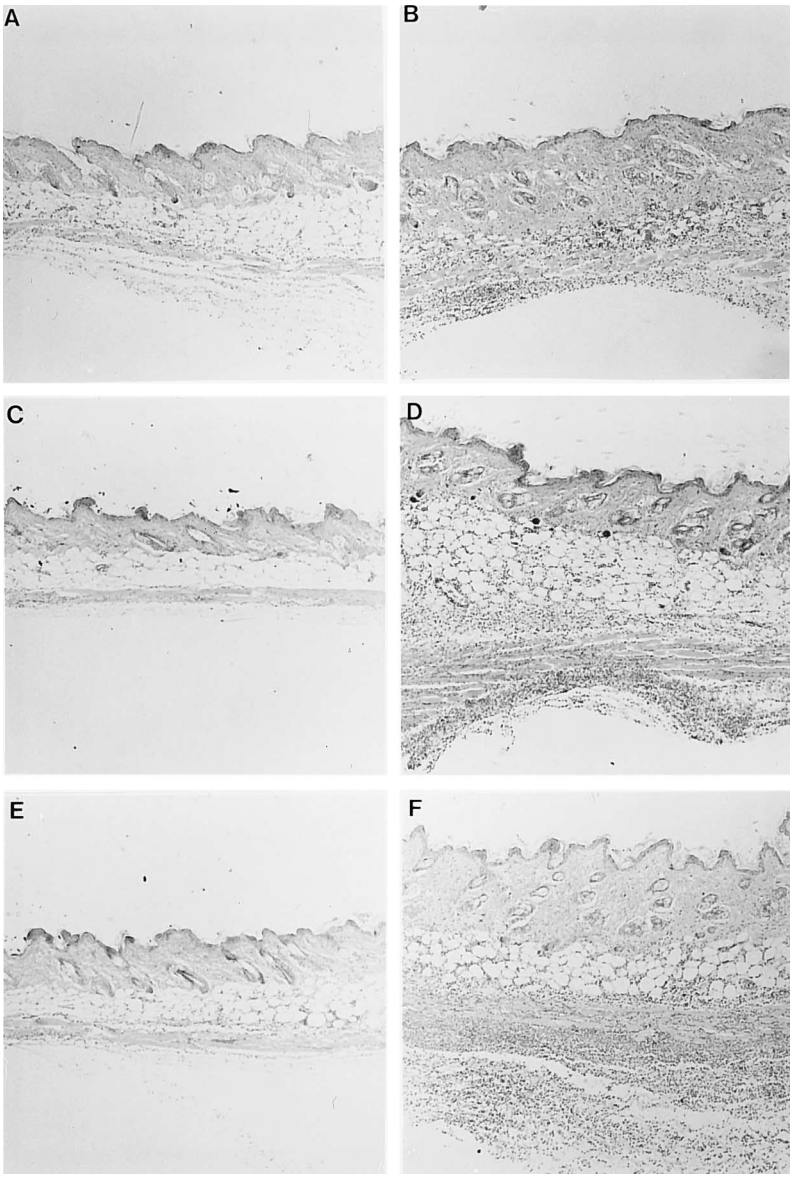

FIG. 1. Dose-dependent dermal inflammatory responses (day 1) to the *T. pallidum* 47-kDa lipopeptide in mice (B, D, and F). The corresponding nonacylated hexapeptide was used as a control (A, C, and E). Doses included 7.1 (A and B), 17.9 (C and D), and 35.8 (E and F) nmol of lipopeptide or hexapeptide. Magnification at the film plane (using a 4× objective) was ×16.5.

described by DeOgny et al. (16). Endotoxin contaminations in synthetic lipopeptide and peptide preparations were below the limits of detection (i.e., <1.2 pg) by the *Limulus* amoebocyte lysate gelation assay (E-Toxate; Sigma Chemical Co., St. Louis, Mo.).

Formulations for dermal injections. Rough LPS (from *Salmonella minnesota* R5 [Rc mutant]; molecular weight, ca. 4,000; Sigma Chemical Co. L-8893) was used as a positive control for dermal inflammation. Peptides, lipopeptides, and LPS were dissolved or suspended at various amounts in sterile phosphate-buffered saline prepared with endotoxin-free water.

Animal injections and histopathology. Fur was clipped from the backs of either 8-week-old BALB/c mice or adult New Zealand White male rabbits. Animals were injected intradermally at multiple sites (for mice, 2 sites per animal and 0.05 ml per site; for rabbits, up to 12 sites per animal and 0.1 ml per site); the injection sites were adequately spaced to ensure that lesion development was not influenced by adjacent sites. Injection sites were examined daily and scored for gross parameters (e.g., erythema, swelling, induration, and eschar formation). At various times, animals were euthanized (mice by CO_2 inhalation and rabbits by lethal injection of pentobarbital), and full-thickness skin specimens were harvested and fixed in 10% neutral-buffered formalin. Tissues were then embedded in paraffin and processed and sectioned by standard methods. The tissues were stained with hematoxylin and eosin; specimens were coded and examined in blinded fashion. Slides were photographed using an Olympus BH2 microscope configured with a PM-10ADS automatic photomicrographic system.

RESULTS AND DISCUSSION

Dose-response experiments were performed by intradermally injecting mice with 10 (7.1-nmol)-, 25 (17.9-nmol)-, or 50 (35.8-nmol)-μg amounts of the synthetic lipopeptide corresponding to the 47-kDa lipoprotein of *T. pallidum* (47-kDa lipopeptide) and by performing histopathological analyses over a 5-day interval. At day 1 postinjection, no dermal lesions were grossly discernible at sites injected with the 47-kDa lipopeptide preparations (not shown). However, at this time point, histopathology revealed that the 47-kDa lipopeptide promoted a dose-dependent acute dermatitis which consisted chiefly of neutrophils diffusely infiltrating the deep dermis and underlying adipose and muscle (Fig. 1B, D, and F). The inflammatory

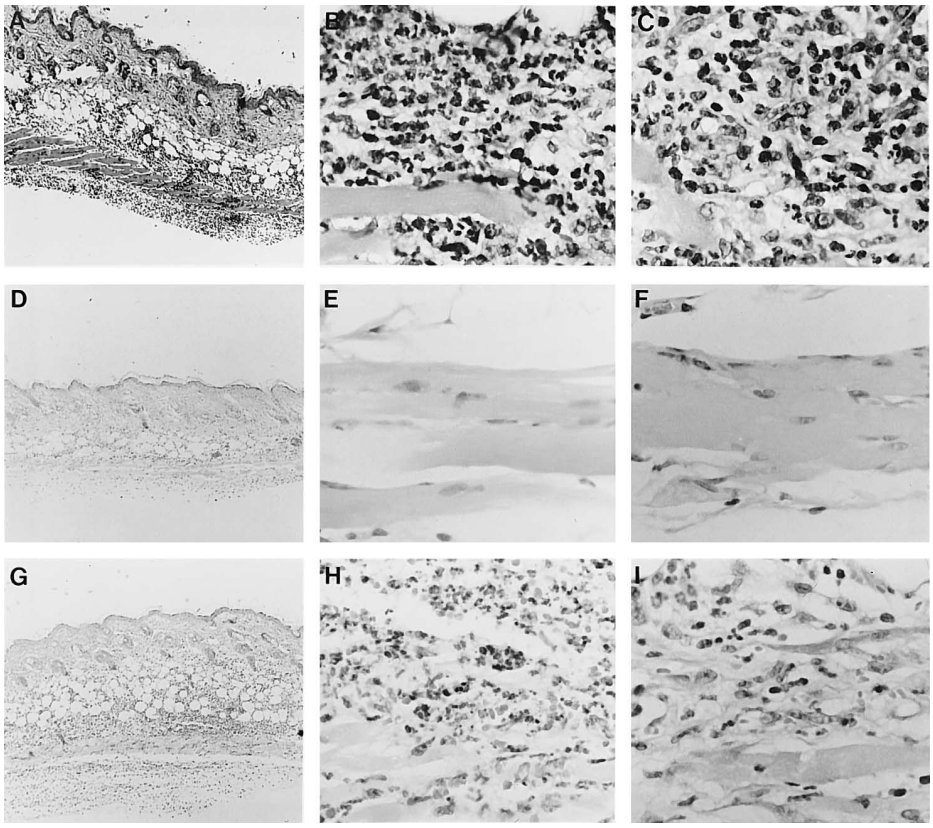

FIG. 2. Inflammatory responses in the deep dermis at days 2 (A, B, D, E, G, and H) and 5 (C, F, and I) postinjection of the 47-kDa lipopeptide (35.8 nmol; 50 μg) in mice (G, H, and I). An equimolar quantity (25 μg) of the corresponding nonacylated peptide was used as a control (D, E, and F). LPS (25 μg; 6.25 nmol) was used as a positive control for dermal inflammation (A, B, and C). Magnifications at the film plane: ×16.5 (4× objective) for panels A, D, and G; ×165 (40× objective) for panels B, C, E, F, H, and I.

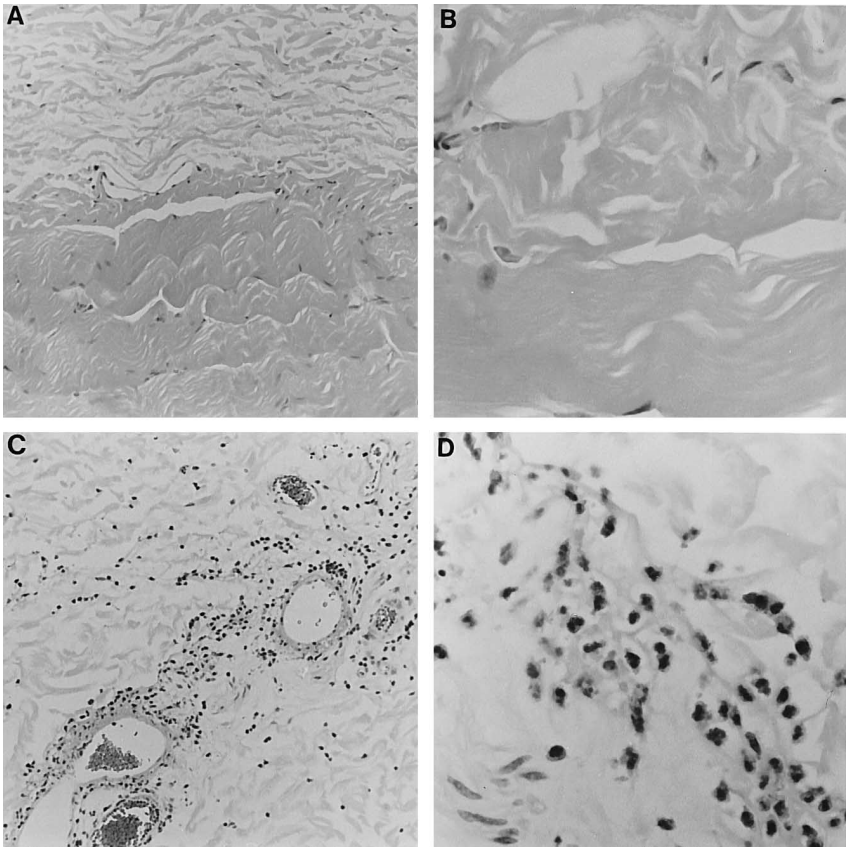

FIG. 3. Inflammatory responses in the deep dermis (day 1) to the *T. pallidum* 47-kDa lipopeptide (35.8 nmol; 50 μg) in rabbits (C and D). An equimolar quantity (25 μg) of the corresponding nonacylated peptide was used as a control (A and B). Magnifications at the film plane: ×41 (10× objective) for panels A and C and ×165 (40× objective) for panels B and D.

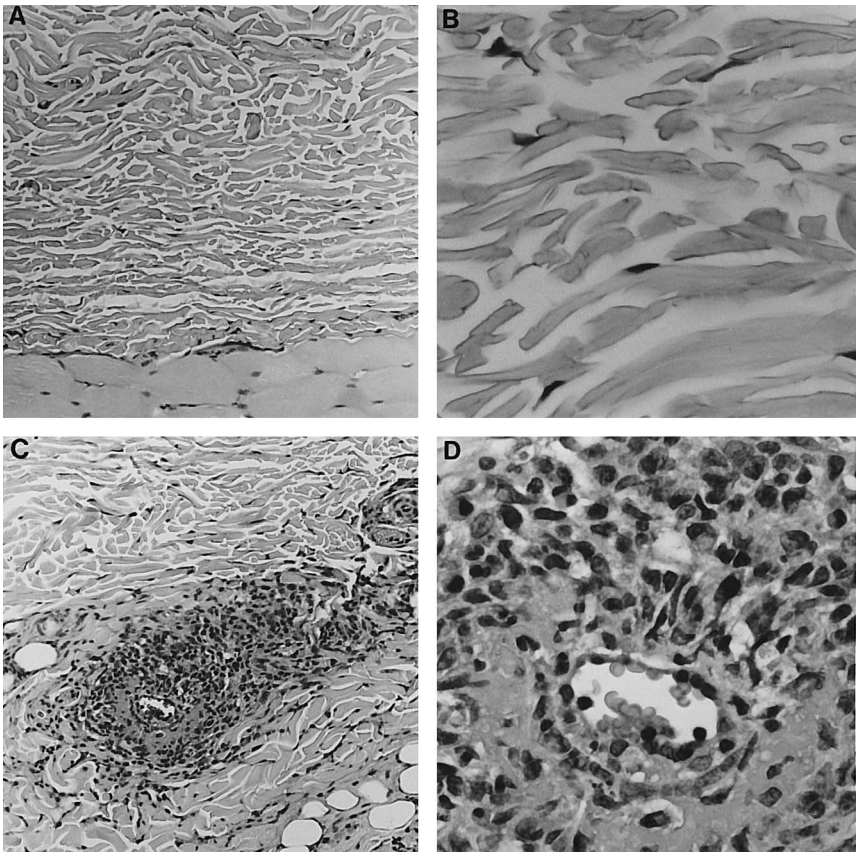

FIG. 4. Inflammatory responses in the deep dermis (day 7) to the *T. pallidum* 47-kDa lipopeptide (35.8 nmol; 50 μg) in rabbits (C and D). An equimolar quantity (25 μg) of the corresponding nonacylated peptide was used as a control (A and B). Magnifications at the film plane: ×41 (10× objective) for panels A and C and ×165 (40× objective) for panels B and D.

response varied from mild at sites injected with 10 μg (Fig. 1A and B) to more marked at those injected with 50 μg (Fig. 1E and F). At day 2, sites injected with the 47-kDa lipopeptide were slightly erythematous and indurated (not shown). Histologically, neutrophils predominated (Fig. 2G and H for 50-μg dose; not shown for 10- and 25-μg doses), and focal necrosis and hemorrhage were also evident (not shown). By day 5, the cellular infiltrates had undergone a transition from an acute to a chronic inflammatory response consisting principally of lymphocytes and macrophages along with a few scattered neutrophils and plasma cells (Fig. 2I). Identical molar quantities of the nonacylated hexapeptide failed to induce grossly apparent lesions (not shown), and cellular infiltrates at the various time points were either inapparent (Fig. 1A, C, and E) or minimal (Fig. 2D, E, and F). The marked difference in the in vivo inflammatory activities of the lipidated and nonlipidated peptides was consistent with studies demonstrating that acylation of native spirochetal lipoproteins, as well as their synthetic analogs, is essential for immune cell activation in vitro (1, 16, 37, 56).

In the same experiment, mice were also challenged intradermally with LPS, although it was necessary to utilize a smaller molar quantity (25 μg; 6.25 nmol) than the quantity of lipopeptide because of the greater toxicity of LPS. The day 1 and day 2 lesions induced by LPS were erythematous, raised, and centrally indurated (not shown). The neutrophilic infiltrates induced by LPS in the deep dermis and subjacent fat and muscle were intense (Fig. 2A and B); thrombosis of small vessels was also evident. At day 5, the gross dermal lesions induced by LPS were larger and more erythematous than day 2 lesions (not shown). By day 5, the inflammatory response was still marked and consisted primarily of lymphocytes, macrophages, and neutrophils (Fig. 2C). Overall, the inflammatory response to 6.25 nmol of LPS was more intense than the response to 35.8 nmol of the lipopeptide, consistent with in vitro studies which have shown that LPS is a substantially more potent macrophage activator than synthetic lipopeptides (16, 37). The lesser potency of the lipopeptide also is in agreement with the fact that, even during their spirochetemic phases, syphilis and Lyme disease typically lack the septic pathophysiology induced by LPS.

Whereas inbred mice provided a convenient system for the initial study of the inflammatory properties of the treponemal lipopeptide in vivo, rabbits are the preferred animal model for venereal syphilis (43, 44). Rabbits challenged intradermally with virulent *T. pallidum* develop primary lesions analogous to those of human chancres; lesions develop over a 2-week period (depending on the size of the inoculum) and ultimately resolve spontaneously, at which time the animals remain latently infected (8). In addition, the histopathology of the dermal response to *T. pallidum* in the rabbit model of syphilis has been well characterized (13, 43, 44, 46). To test whether the intradermal response in rabbits to the synthetic treponemal lipopeptide resembled that occurring during experimental syphilis, rabbits were injected intradermally with 50 μg of the 47-kDa lipopeptide. On day 1, only slight erythema was observed at sites injected with the 47-kDa lipopeptide (not shown); histopathologically, edema, vascular congestion, and a mild

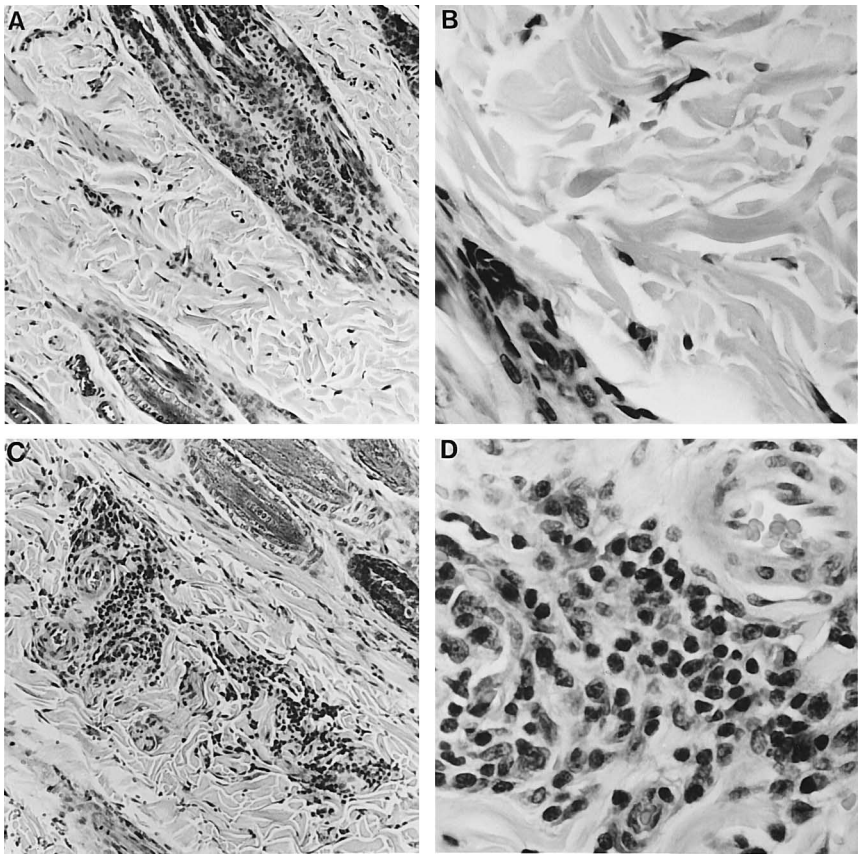

FIG. 5. Inflammatory responses in the upper dermis (day 14) to the *T. pallidum* 47-kDa lipopeptide (35.8 nmol; 50 μg) in rabbits (C and D). An equimolar quantity (25 μg) of the corresponding nonacylated peptide was used as a control (A and B). Magnifications at the film plane: ×41 (10× objective) for panels A and C and ×165 (40× objective) for panels B and D.

perivascular heterophilic infiltrate in the deep dermis were noted (Fig. 3C and D). By day 7, erythema had expanded slightly from the initial site of injection (not shown); histopathologic findings included vasculitis of deep dermal vessels and perivascular infiltrates of lymphocytes, macrophages, and small numbers of heterophils (Fig. 4C and D). Scattered deep dermal vessels were thrombosed. After 14 days, gross erythema remained modest (not shown); however, histopathologic analysis revealed a superficial and deep perivascular mononuclear infiltrate consisting of lymphocytes, plasma cells, and small numbers of macrophages (Fig. 5C and D for upper dermis; data not shown for deep dermis). Heterophils were not represented in this chronic lesion. Of note, the presence of perivascular mononuclear infiltrates containing plasma cells is a histopathologic feature distinctive of syphilis (26, 45). An equimolar quantity of the nonacylated hexapeptide injected intradermally into rabbits elicited neither gross (not shown) nor microscopic (Fig. 3A and B, 4A and B, and 5A and B) inflammation. LPS (25 μg) injected intradermally into rabbits evoked a more intense acute inflammatory response similar to that observed with mice (not shown).

Several aspects of these findings for rabbits are noteworthy. First, Sell et al. (46) reported that the early (less than 24-h) inflammatory response to intradermal inoculation of normal rabbits with *T. pallidum* is characterized by a perivascular and diffuse dermal infiltrate of heterophils, analogous to observations made in the present study with the 47-kDa lipopeptide. Second, by day 7, we observed a shift toward mononuclear infiltrates in lipopeptide-induced lesions, consistent with the typical cellular response to *T. pallidum* in rabbit dermal tissue (44, 46). Third, 14 days postinjection with *T. pallidum*, Sell et al. (44) noted a more-pronounced mononuclear cell infiltrate; this included enhanced perivasculitis, more inflammatory cells in the upper dermis, and extensive lymphocytic infiltration surrounding the hair follicles. This course was comparable to that observed in the present study using the 47-kDa lipopeptide. It also seems particularly significant that, as in the case of prior studies with rabbits inoculated with virulent *T. pallidum* (13, 43, 44, 46), histopathologic patterns in response to the 47-kDa lipopeptide in rabbit dermal tissue had features in common with those described for human primary and secondary syphilis (19, 23, 26, 51), including the presence of plasma cells. Finally, perivasculitis and thrombosis induced in the rabbit dermis were consistent with prior studies of Riley et al. (39, 40) demonstrating that *T. pallidum* or the 47-kDa treponemal lipoprotein activates endothelial cells in vitro to upregulate the expression of leukocyte adhesion molecules (e.g., intercellular adhesion molecule 1) involved in leukocyte homing as well as procoagulant activity (tissue factor) active in the extrinsic pathway of coagulation.

It also was of interest to examine the dermal response elicited by a synthetic analog of a *B. burgdorferi* lipoprotein (i.e., OspA). Mice were injected intradermally with either 10, 25, or 50 μg of the OspA lipopeptide and were monitored for 13 days, a time point which, according to the above studies (Fig. 1 and 2), was predicted to be beyond the transition from an

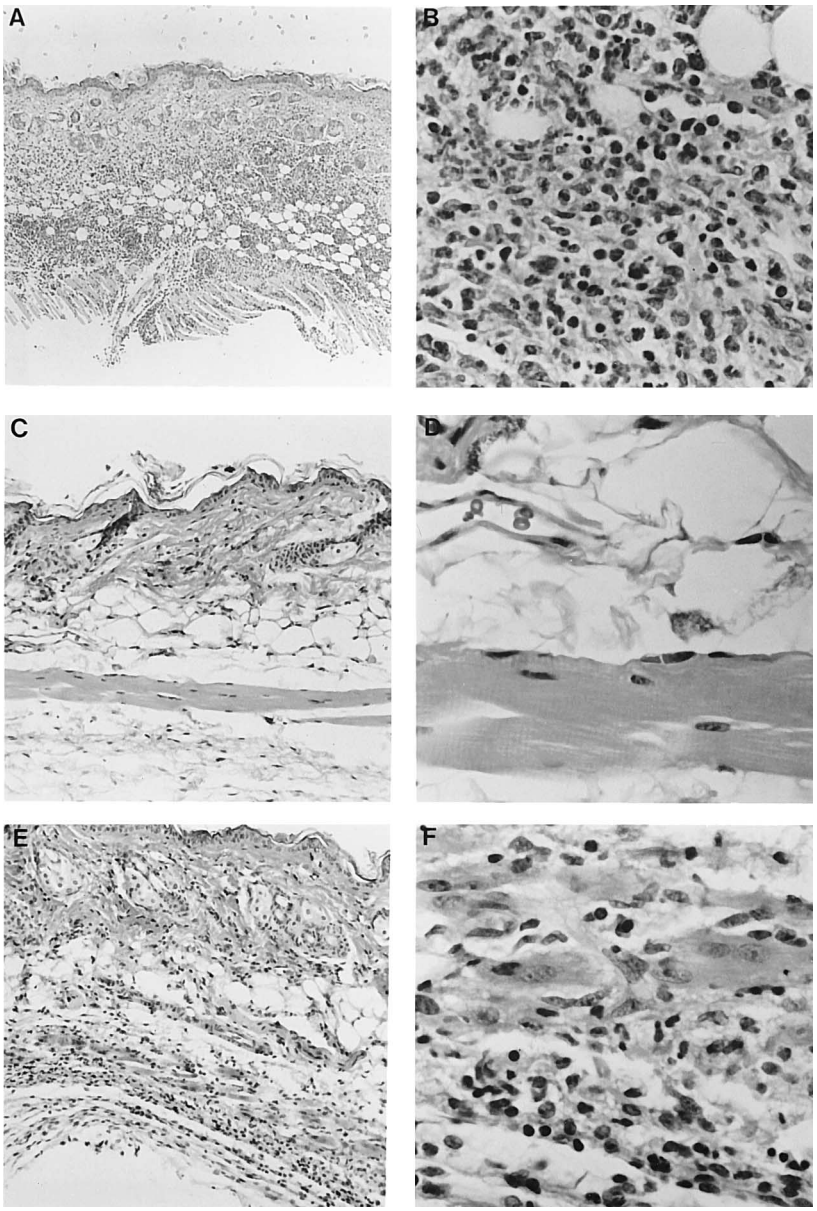

FIG. 6. Inflammatory responses in the deep dermis (day 5) to the OspA lipopeptide (35.8 nmol; 50 μg) in mice (E and F). An equimolar quantity (25 μg) of the corresponding nonacylated peptide was used as a control (C and D). LPS (25 μg; 6.25 nmol) was used as a positive control for dermal inflammation (A and B). Magnifications at the film plane: ×16.5 (4× objective) for panel A, ×41 (10× objective) for panels C and E, and ×165 (40× objective) for panels B, D, and F.

acute to a chronic inflammatory response. Results obtained at days 1 and 2 postinjection of the OspA lipopeptide grossly and histopathologically were similar to those obtained with the 47-kDa lipopeptide (not shown). At day 5, mouse dermal tissues demonstrated a dose-dependent response consisting of a diffuse superficial and deep dermatitis which contained a preponderance of mononuclear cells and scattered neutrophils (Fig. 6E and F for a 50-μg dose; not shown for 10- and 25-μg doses), whereas the nonacylated OspA hexapeptide failed to induce an inflammatory response (Fig. 6C and D). By day 13, OspA lipopeptide-induced inflammation was resolving, with scattered mononuclear cells remaining (Fig. 7E and F); however, the response to LPS (25 μg) remained very intense in both the superficial and deep dermis and consisted of lymphocytes, macrophages, and neutrophils (Fig. 7A and B). Taken together, histopathology elicited by the OspA lipopeptide in dermal tissues of mice was consistent with findings of other histopathological studies of *B. burgdorferi* infection in both mice and humans (2, 3, 5, 18).

The combined observations from this study support the contention that the spirochetal lipoproteins/lipopeptides serve as inflammatory mediators in syphilis and Lyme disease (1, 16, 28, 29, 36, 37, 39, 49, 56). While there are several mechanisms by which they could exert these activities, we propose that as spirochetes invade and disseminate within tissues, some are engulfed by macrophages (25, 41). In addition to processing antigens for presentation of peptides to T lymphocytes in the context of class II major histocompatibility complex molecules, biologically active lipopeptides may be liberated from intact lipoproteins. Alternatively, lipoproteins may be shed from

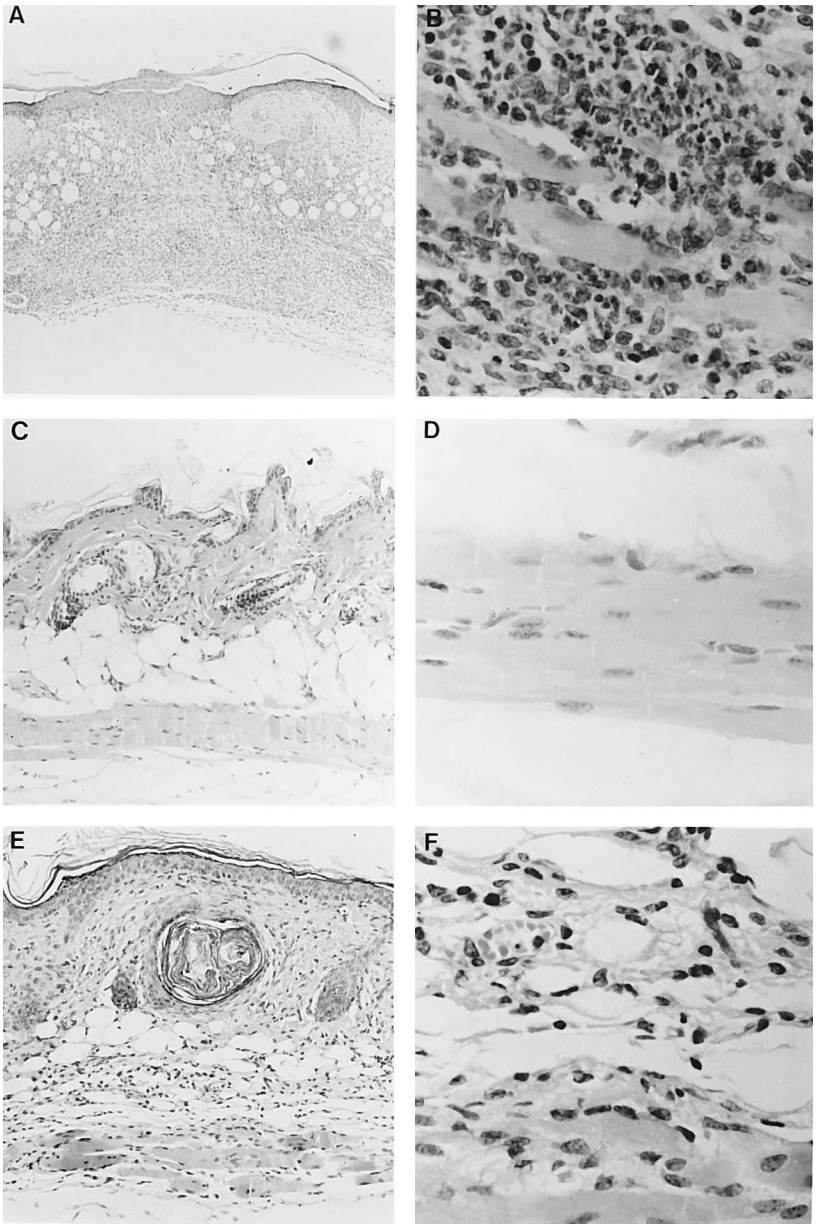

FIG. 7. Inflammatory responses in the deep dermis (day 13) to the OspA lipopeptide (35.8 nmol; 50 μg) in mice (E and F). An equimolar quantity (25 μg) of the corresponding nonacylated peptide was used as a control (C and D). LPS (25 μg; 6.25 nmol) was used as a positive control for dermal inflammation (A and B). Magnifications at the film plane: ×16.5 (4× objective) for panel A, ×41 (10× objective) for panels C and E, and ×165 (40× objective) for panels B, D, and F.

dead or dying organisms. Cytokines (e.g., tumor necrosis factor alpha, interleukin-1β, and interleukin 6) induced in the macrophage, an immune effector cell which is particularly sensitive to activation by lipoproteins/lipopeptides (37), could amplify specific cellular responses as well as recruit additional effector cells to local sites of infection (e.g., by upregulating expression of leukocyte adhesion molecules on vascular endothelium). Recruitment of large numbers of immunologically uncommitted immune effector cells to these sites (33) could help explain why the lesions induced by the lipopeptides have histopathological similarities to those of acquired or experimental syphilis and Lyme disease. Some organisms escape these clearance mechanisms (27), thereby giving rise to persistent infection, which provides the continuous antigenic stimulus for disease progression. An attractive feature of this scenario is that spirochetal lipoproteins need not be surface-exposed to exert these biological effects. Virtually all of the lipoproteins of *T. pallidum* appear to be associated with the cytoplasmic rather than the outer membrane (14, 15), and we have reported that in *B. burgdorferi* substantial portions of OspA and OspB are also subsurface (10, 35). Finally, although the 47-kDa and OspA analogs were used in the current study as representative lipopeptides, we and others have shown that lipoproteins/lipopeptides with different amino acid sequences have cytokine stimulatory properties (1, 16, 21, 28, 29, 36, 37, 49). Thus, it is plausible that the diverse lipoproteins in each spirochete (9, 12) act cumulatively to promote inflammation.

The findings reported here also have implications for vaccine development. Despite their inflammatory properties, the spirochetal lipopeptides displayed relatively low toxicity in

comparison with either LPS (this study) or complete Freund's adjuvant (unpublished observations). Similar findings were noted by Wiedemann et al. (57) using a synthetic analog of Braun's lipoprotein of *E. coli* injected subcutaneously into mice. Moreover, the propensities for the spirochetal lipopeptides to recruit activated macrophages and lymphocytes in the absence of significant tissue toxicity are desired features of immune adjuvants (30, 52, 53). The need to develop new adjuvants for use in human vaccines (53) provides impetus for further investigations of the spirochetal lipopeptides as novel, nontoxic adjuvants. Studies employing analogs of Braun's lipoprotein conjugated to immunodominant B-cell or T-cell epitopes have shown that such constructs can enhance specific immune responses (7, 17, 24, 38, 58).

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REFERENCES

1. **Akins, D. R., B. K. Purcell, M. Mitra, M. V. Norgard, and J. D. Radolf.** 1993. Lipid modification of the 17-kilodalton membrane immunogen of *Treponema pallidum* determines macrophage activation as well as amphiphilicity. Infect. Immun. **61:**1202–1210.
2. **Barthold, S. W., M. S. deSouza, J. L. Janotka, A. L. Smith, and D. H. Persing.** 1993. Chronic Lyme borreliosis in the laboratory mouse. Am. J. Pathol. **143:**959–971.
3. **Barthold, S. W., D. H. Persing, A. L. Armstrong, and R. A. Peeples.** 1991. Kinetics of *Borrelia burgdorferi* dissemination and evolution of disease after intradermal inoculation of mice. Am. J. Pathol. **139:**263–273.
4. **Beck, G., J. L. Benach, and G. S. Habicht.** 1990. Isolation, preliminary chemical characterization, and biological activity of *Borrelia burgdorferi* peptidoglycan. Biochem. Biophys. Res. Commun. **167:**89–95.
5. **Berger, B. W.** 1989. Dermatologic manifestations of Lyme disease. Rev. Infect. Dis. **11**(Suppl. 6)**:**S1475–S1481.
6. **Bergstrom, S., V. G. Bundoc, and A. G. Barbour.** 1989. Molecular analysis of linear plasmid-encoded major surface proteins, OspA and OspB, of the Lyme disease spirochaete *Borrelia burgdorferi*. Mol. Microbiol. **3:**479–486.
7. **Bessler, W. G., B. Suhr, H.-J. Buhring, C. P. Muller, K.-H. Wiesmuller, G. Becker, and G. Jung.** 1985. Specific antibodies elicited by antigen covalently linked to a synthetic adjuvant. Immunobiology **170:**239–244.
8. **Bishop, N. H., and J. N. Miller.** 1976. Humoral immunity in experimental syphilis. I. The demonstration of resistance conferred by passive immunization. J. Immunol. **117:**191–196.
9. **Brandt, M. E., B. S. Riley, J. D. Radolf, and M. V. Norgard.** 1990. Immunogenic integral membrane proteins of *Borrelia burgdorferi* are lipoproteins. Infect. Immun. **58:**983–991.
10. **Brusca, J. F., A. W. McDowall, M. V. Norgard, and J. D. Radolf.** 1991. Localization of outer surface proteins A and B in both the outer membrane and intracellular compartments of *Borrelia burgdorferi*. J. Bacteriol. **173:** 8004–8008.
11. **Campagnari, A. A., L. M. Wild, G. E. Griffiths, R. J. Karalus, M. A. Wirth, and S. M. Spinola.** 1991. Role of lipooligosaccharides in experimental dermal lesions caused by *Haemophilus ducreyi*. Infect. Immun. **59:**2601–2608.
12. **Chamberlain, N. R., M. E. Brandt, A. L. Erwin, J. D. Radolf, and M. V. Norgard.** 1989. Major integral membrane protein immunogens of *Treponema pallidum* are proteolipids. Infect. Immun. **57:**2872–2877.
13. **Collart, P., P. Franceschini, and P. Durel.** 1971. Experimental rabbit syphilis. Br. J. Vener. Dis. **47:**389–400.
14. **Cox, D. L., D. R. Akins, S. F. Porcella, M. V. Norgard, and J. D. Radolf.** *Treponema pallidum* in gel microdroplets: a novel strategy for investigation of treponemal molecular architecture. Mol. Microbiol., in press.
15. **Cox, D. L., P. Chang, A. McDowall, and J. D. Radolf.** 1992. The outer membrane, not a coat of host proteins, limits the antigenicity of virulent *Treponema pallidum*. Infect. Immun. **60:**1076–1083.
16. **DeOgny, L., B. C. Pramanik, L. L. Arndt, J. D. Jones, J. Rush, C. A. Slaughter, J. D. Radolf, and M. V. Norgard.** 1994. Solid-phase synthesis of biologically active lipopeptides as analogs for spirochetal lipoproteins. Pept. Res. **7:**91–97.
17. **Deres, K., H. Schild, K.-H. Wiesmuller, G. Jung, and H.-G. Rammensee.** 1989. In vivo priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine. Nature (London) **342:**561–564.
18. **Duray, P. H.** 1989. Clinical pathologic correlations of Lyme disease. Rev. Infect. Dis. **11S:**S1487–S1493.
19. **Engelkens, H. J. H., F. J. W. ten Kate, J. Judanarso, V. D. Vuzevski, J. B. H. J. van Lier, J. C. J. Godschalk, J. J. Van der Sluis, and E. Stolz.** 1993. The localisation of treponemes and characterisation of the inflammatory infiltrate in skin biopsies from patients with primary or secondary syphilis, or early infectious yaws. Genitourin. Med. **69:**102–107.
20. **Hardy, P. H., Jr., and J. Levin.** 1983. Lack of endotoxin in *Borrelia hispanica* and *Treponema pallidum*. Proc. Soc. Exp. Biol. Med. **174:**47–52.
21. **Hauschildt, S., P. Hoffmann, H. U. Beuscher, G. Dufhues, P. Heinrich, K.-H. Wiesmuller, G. Jung, and W. G. Bessler.** 1990. Activation of bone marrow-derived mouse macrophages by bacterial lipopeptide: cytokine production, phagocytosis, and Ia expression. Eur. J. Immunol. **20:**63–68.
22. **Hoffmann, P., S. Heinle, U. F. Schade, H. Loppnow, A. J. Ulmer, H.-D. Flad, G. Jung, and W. G. Bessler.** 1988. Stimulation of human and murine adherent cells by bacterial lipoprotein and synthetic lipopeptide analogues. Immunobiology **177:**158–170.
23. **Jeerapaet, P., and A. B. Ackerman.** 1973. Histologic patterns of secondary syphilis. Arch. Dermatol. **107:**373–377.
24. **Lex, A., K.-H. Wiesmuller, G. Jung, and W. G. Bessler.** 1986. A synthetic analogue of *Escherichia coli* lipoprotein, tripalmitoyl pentapeptide, constitutes a potent immune adjuvant. J. Immunol. **137:**2676–2681.
25. **Lukehart, S. A.** 1983. Macrophages and host resistance, p. 349–364. *In* R. F. Schell and D. M. Musher (ed.), Pathogenesis and immunology of treponemal infection. Marcel Dekker, New York.
26. **Lukehart, S. A., and K. K. Holmes.** 1994. Syphilis, p. 726–737. *In* K. J. Isselbacher, E. Braunwald, J. Wilson, J. B. Martin, A. S. Fauci, and D. L. Kasper (ed.), Harrison's principles of internal medicine. McGraw Hill, Inc., New York.
27. **Lukehart, S. A., J. M. Shaffer, and S. A. Baker-Zander.** 1992. A subpopulation of *Treponema pallidum* is resistant to phagocytosis: possible mechanisms of persistence. J. Infect. Dis. **166:**1449–1453.
28. **Ma, Y., K. P. Seiler, K.-F. Tai, L. Yang, M. Woods, and J. J. Weis.** 1994. Outer surface lipoproteins of *Borrelia burgdorferi* stimulate nitric oxide production by the cytokine-inducible pathway. Infect. Immun. **62:**3663–3671.
29. **Ma, Y., and J. J. Weis.** 1993. *Borrelia burgdorferi* outer surface lipoproteins OspA and OspB possess B-cell mitogenic and cytokine-stimulatory properties. Infect. Immun. **61:**3843–3853.
30. **McGhee, J. R., J. J. Farrar, S. M. Michalek, S. E. Mergenhagen, and D. L. Rosenstreich.** 1979. Cellular requirements for lipopolysaccharide adjuvanticity. A role for both T lymphocytes and macrophages for *in vitro* responses to particulate antigens. J. Exp. Med. **149:**793–807.
31. **Morrison, D. C., R. Danner, C. A. Dinarello, R. S. Munford, C. Natanson, M. Pollack, J. J. Spitzer, R. J. Ulevitch, S. N. Vogel, and E. McSweegan.** 1994. Bacterial endotoxins and pathogenesis of gram negative infections: current status and future direction. J. Endotoxin Res. **1:**71–83.
32. **Ohanian, S. H., and J. H. Schwab.** 1967. Persistence of group A streptococcal cell walls related to chronic inflammation of rabbit dermal connective tissue. J. Exp. Med. **125:**1137–1148.
33. **Picker, L. J., and E. C. Butcher.** 1992. Physiological and molecular mechanisms of lymphocyte homing. Annu. Rev. Immunol. **10:**561–591.
34. **Radolf, J. D., J. S. Brusca, M. E. Brandt, and M. V. Norgard.** 1992. Spirochete molecular architecture and Lyme disease pathogenesis, p. 119–134. *In* S. E. Schutzer (ed.), Lyme disease: molecular and immunologic approaches. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
35. **Radolf, J. D., M. S. Goldberg, K. Bourell, J. D. Jones, and M. V. Norgard.** Unpublished data.
36. **Radolf, J. D., M. V. Norgard, M. E. Brandt, R. D. Isaacs, P. A. Thompson, and B. Beutler.** 1991. Lipoproteins of *Borrelia burgdorferi* and *Treponema pallidum* activate cachectin/tumor necrosis factor synthesis: analysis using a CAT reporter construct. J. Immunol. **147:**1968–1974.
37. **Radolf, J. R., L. L. Arndt, D. R. Akins, L. L. Curetty, M. E. Levi, Y. Shen, L. S. Davis, and M. V. Norgard.** *Treponema pallidum* and *Borrelia burgdorferi* lipoproteins and synthetic lipopeptides activate monocytes/macrophages. J. Immunol., in press.
38. **Reitermann, A., J. Metzger, K.-H. Wiesmüller, G. Jung, and W. G. Bessler.** 1989. Lipopeptide derivatives of bacterial lipoprotein constitute potent immune adjuvants combined with or covalently coupled to antigen or hapten. Biol. Chem. Hoppe-Seyler **370:**343–352.
39. **Riley, B. S., N. Oppenheimer-Marks, E. J. Hansen, J. D. Radolf, and M. V. Norgard.** 1992. Virulent *Treponema pallidum* activates human vascular endothelial cells. J. Infect. Dis. **165:**484–493.
40. **Riley, B. S., N. Oppenheimer-Marks, J. D. Radolf, and M. V. Norgard.** 1994. Virulent *Treponema pallidum* promotes adhesion of leukocytes to human vascular endothelial cells. Infect. Immun. **62:**4622–4625.

41. **Rittig, M. G., T. Haupl, and G. R. Burmester.** 1994. Coiling phagocytosis: a way for MHC class I presentation of bacterial antigens. Int. Arch. Allergy Immunol. **103:**4–10.
42. **Schouls, L. M., R. Mout, J. Dekker, and J. D. A. Van Embden.** 1989. Characterization of lipid-modified immunogenic proteins of *Treponema pallidum* expressed in *Escherichia coli*. Microb. Pathog. **7:**175–188.
43. **Sell, S.** 1983. Histopathology and immunopathology of experimental syphilis, p. 297–313. *In* R. F. Schell and D. M. Musher (ed.), Pathogenesis and immunology of treponemal infection. Marcel Dekker, New York.
44. **Sell, S., D. Gamboa, S. A. Baker Zander, S. A. Lukehart, and J. N. Miller.** 1980. Host response to *Treponema pallidum* in intradermally-infected rabbits: evidence for persistence of infection at local and distant sites. J. Invest. Dermatol. **75:**470–475.
45. **Sell, S., and S. J. Norris.** 1983. The biology, pathology, and immunology of syphilis. Int. Rev. Exp. Pathol. **24:**203–276.
46. **Sell, S., J. Salman, and S. J. Norris.** 1985. Reinfection of chancre-immune rabbits with *Treponema pallidum*. I. Light and immunofluorescence studies. Am. J. Pathol. **118:**248–255.
47. **Steere, A. C.** 1989. Lyme disease. N. Engl. J. Med. **321:**586–597.
48. **Szczepanski, A., and J. L. Benach.** 1991. Lyme borreliosis: host responses to *Borrelia burgdorferi*. Microbiol. Rev. **55:**21–34.
49. **Tai, K.-F., Y. Ma, and J. J. Weis.** 1994. Normal human B lymphocytes and mononuclear cells respond to the mitogenic and cytokine-stimulatory activities of *Borrelia burgdorferi* and its lipoprotein OspA. Infect. Immun. **62:**520–528.
50. **Takayama, K., R. J. Rothenberg, and A. G. Barbour.** 1987. Absence of lipopolysaccharide in the Lyme disease spirochete, *Borrelia burgdorferi*. Infect. Immun. **55:**2311–2313.
51. **Tosca, A., J. Lehou, M. Hatjivasiliou, A. Varelzidis, and J. D. Stratigos.** 1988. Infiltrate of syphilitic lesions before and after treatment. Genitourin. Med. **64:**289–293.
52. **Verma, J. N., M. Rao, S. Amselem, U. Krzych, C. R. Alving, S. J. Green, and N. M. Wassef.** 1992. Adjuvant effects of liposomes containing lipid A: enhancement of liposomal antigen presentation and recruitment of macrophages. Infect. Immun. **60:**2438–2444.
53. **Warren, H. S., F. R. Vogel, and L. A. Chedid.** 1986. Current status of immunological adjuvants. Annu. Rev. Immunol. **4:**369–388.
54. **Weigel, L. M., M. E. Brandt, and M. V. Norgard.** 1992. Analysis of the N-terminal region of the 47-kilodalton integral membrane lipoprotein of *Treponema pallidum*. Infect. Immun. **60:**1568–1576.
55. **Weigel, L. M., J. D. Radolf, and M. V. Norgard.** 1994. The 47-kDa major lipoprotein immunogen of *Treponema pallidum* is a penicillin-binding protein with carboxypeptidase activity. Proc. Natl. Acad. Sci. USA **91:**11611–11615.
56. **Weis, J. J., Y. Ma, and L. F. Erdile.** 1994. Biological activities of native and recombinant *Borrelia burgdorferi* outer surface protein A: dependence on lipid modification. Infect. Immun. **62:**4632–4636.
57. **Wiedemann, F., R. Link, K. Pumpe, U. Jacobshagen, H. E. Schaefer, K.-H. Wiesmüller, R.-P. Hummel, G. Jung, W. Bessler, and T. Böltz.** 1991. Histopathological studies on the local reactions induced by complete Freund's adjuvant (CFA), bacterial lipopolysaccharide (LPS), and synthetic lipopeptide (P_3C) conjugates. J. Pathol. **164:**265–271.
58. **Wiesmüller, K.-H., G. Jung, and G. Hess.** 1989. Novel low-molecular weight synthetic vaccine against foot-and-mouth disease containing a potent B-cell and macrophage activator. Vaccine **7:**29–33.